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(54) Title: COMPOSITIONS USEFUL AS LIGANDS FOR THE FORMYL PEPTIDE RECEPTOR LIKE 1 RECEPTOR AND METHODS OF USE THEREOF

(57) Abstract: The inventors have discovered that a CK $\beta$ 8-1 truncation variant, CK $\beta$ 8-1 (25-116), is a bifunctional ligand for two distinct GPCRs, chemokine receptor CCR1 and formyl peptide receptor like 1 (FPRL1). Hence, the inventors have discovered that, in addition to its functional activity on CCR1, CK $\beta$ 8-1(25-116) is also a functional ligand for the GPCR receptor FPRL1 that is involved in inflammatory reactions and innate immunity by recruiting monocytes and neutrophils. In addition, the inventors have discovered an alternatively spliced exon of CK $\beta$ 8-1, named SHAAGtide. SHAAGtide, along with its parent chemokine CK $\beta$ 8-1(25-116), is fully functional on both monocytes and neutrophils that are known to express FPRL1.

**COMPOSITIONS USEFUL AS LIGANDS FOR THE FORMYL PEPTIDE  
RECEPTOR LIKE 1 RECEPTOR AND METHODS OF USE THEREOF.**

5 **FIELD OF THE INVENTION**

The invention relates to compositions useful as ligands for the Formyl Peptide Receptor Like 1 receptor and methods of use thereof.

**BACKGROUND**

10 Chemokines (chemotactic cytokines) act as molecular beacons for the recruitment and activation of T lymphocytes, neutrophils and macrophages, flagging pathogen battlegrounds. Recruitment of leukocytes, the white blood cells responsible for fighting infections depends on gradients of chemokines. Chemokines are a superfamily of small proteins (8-10 KD) that mediate diverse  
15 biological processes including leukocyte trafficking and homing, immunoregulation, hematopoiesis and angiogenesis. To date, 24 chemokine receptors are known. Chemokines play a fundamental role in innate immunity and inflammatory reactions (Baggiolini et al. (1994); Baggiolini et al. (1997); Rollins (1997).) Four subfamilies of chemokines have been described, based on the  
20 distance between the first two conserved cysteine residues: C, CC, CXC, and CX3C. All known chemokines signal through four groups of seven transmembrane receptors which belong to the G protein-coupled receptor and pertussis toxin-sensitive heterotrimeric G proteins of G<sub>i</sub> family: XCR, CCR, CXCR and CX3CR. (Murphy et al. (2000)). Extracellular binding events can  
25 activate specific signal transduction pathways leading to various responses, such as chemotaxis. In the chemokine system, multiple chemokines may activate a single chemokine receptor; for example, the receptor CCR1 ligates the RANTES (regulated on activation normal T cell expressed), MIP-1 $\alpha$  (macrophage inflammatory protein) and MIP-1 $\beta$  chemokines. Likewise, a single chemokine  
30 may activate several receptors (Mantovani (1999)).

Monocytes and neutrophils, which play an important role in the pathogenesis of inflammation and in antigen presentation, respond to chemokines (Lee et al. (2000)). Monocytes express the chemokine receptors CCR1, CCR2, CCR5, CCR8, CXCR2, and CXCR4. (Uguccioni et al. (1995); Weber et al.

(2000)). The ligands MIP-1 $\alpha$  and Monocyte Chemoattractant Protein 1 (MCP1) have been reported as potent monocyte activators *in vitro*. (Fantuzzi et al. (1999).) Neutrophils are crucial during many acute inflammatory responses, and may also play a role in orienting immunity toward Th1 responses. (Bonecchi et al. (1999).)  
5 They mainly respond to some CXC chemokines but do not migrate to most of CC chemokines. Human neutrophils express two high affinity IL-8 receptors, CXCR1 and CXCR2.

The chemokine CK $\beta$ 8, also known as CCL23; hmrp-2a; myeloid progenitor inhibitor factor 1 (MPIF-1); SCYA23 (current nomenclature and Genome ID system),  
10 is a 99-amino acid CC chemokine containing six cysteines. It is constitutively expressed in liver, lung, pancreas, and bone marrow. CK $\beta$ 8 has chemotactic activity on monocytes, dendritic cells, and resting lymphocytes (Forssmann et al. (1997)) and inhibits colony formation of bone marrow-derived low proliferative potential colony-forming cells. (Patel et al. (1997)). CK $\beta$ 8-1, an alternative splicing form of CK $\beta$ 8  
15 that is 116-amino acids in length, has been reported. Both the CK $\beta$ 8 and CK $\beta$ 8-1 mature forms have been assigned as ligands for the CCR1 receptor. (Youn et al. (1998)). Cross-desensitization studies in both monocytes and eosinophils indicate that CK $\beta$ 8-1 binds predominately to the CCR1. Further processing at the NH<sub>2</sub>-terminus of CK $\beta$ 8 results in 76 or 75 residue proteins that are significantly more  
20 active on CCR1 expressing cells (Macphee et al. (1998), Berkhout et al. (2000)).

In addition to the chemokine receptors, neutrophils and monocytes also express the G protein-coupled *N*-formyl peptide receptor (FPR) and its homologue *N*-formyl peptide receptor like 1 (FPRL1). Since the ligands for FPRL1 were unknown when it was originally cloned, FPRL1 was initially defined as an orphan receptor.  
25 (Bao et al. (1992); Murphy et al. (1992); Ye et al. (1992).) It was assigned as a LXA<sub>4</sub> receptor since it binds lipoxin A<sub>4</sub> (Fiore et al. (1994).) In addition, several different peptides/proteins have been reported to bind FPRL1 with low affinity (see Figure 1). A serum amyloid A, a protein secreted during the acute phase of inflammation, has been reported as a medial affinity functional ligand (Su et al. (1999)). A  $\beta$  amyloid  
30 fragment (1-42) and neurotoxic prion peptide 106-126 are also low affinity ligands, indicating that FPRL1 may play a role in neurodegenerative diseases (Le et al. (2001)). Some other low affinity ligands include: peptides derived from HIV envelope proteins (Su et al. (1999), Deng et al. (1999)); and a *Helicobacter pylori*

peptide, Hp(2-20). Some synthetic peptides, such as Trp-Lys-Tyr-Met-Val-D-Met-NH<sub>2</sub> (WKYMVm) and Trp-Lys-Tyr-Met-Val-Met-NH<sub>2</sub> (WKYMVM) ("W peptides 1 and 2"), have been reported as potent ligands for the receptor. (Christophe et al. (2001); Baek et al. (1996)). However, these non-naturally occurring peptides derived  
5 from random hexapeptide libraries have not been shown to be physiologically relevant.

## SUMMARY

The inventors have discovered that the CK $\beta$ 8-1 truncation variant, CK $\beta$ 8-1  
10 (25-116), is involved in inflammatory reactions and innate immunity through its role as a functional ligand for the formyl peptide receptor like 1 receptor (FPRL1). In addition, the inventors have discovered an alternatively spliced exon of CK $\beta$ 8-1, named SHAAGtide, and truncated and other variants of SHAAGtide that, along with CK $\beta$ 8-1 (25-116), are functional on both cells that are known to express FPRL1.  
15 Functional SHAAGtides generate calcium flux upon receptor-ligand binding in leukocytes and attract monocytes, neutrophils, mature dendritic cells (mDCs), and immature dendritic cells (iDCs).

In one embodiment, the invention encompasses SHAAGtides as well as proteins and peptides comprising SHAAGtides, with the exception of CK $\beta$ 8-1 (25-  
20 116). In addition, the invention also includes nucleic acids encoding SHAAGtides, nucleic acids encoding proteins and peptides comprising SHAAGtides, antibodies specifically binding SHAAGtides, and fusion proteins comprising SHAAGtides.

In another embodiment, the invention encompasses compositions comprising SHAAGtides or proteins or peptides comprising a SHAAGtide sequence. Such  
25 compositions include those suitable for administration to a subject to enhance FPRL1 activity.

In a further embodiment, the invention encompasses kits comprising such compositions. Such kits may be assembled to facilitate administration of, for example, pharmaceutical compositions.

30 In another aspect, the invention encompasses methods of treating a subject for a disorder comprising modulating an activity of a FPRL1 receptor by administering a compound comprising of a SHAAGtide or proteins or peptides comprising a SHAAGtide sequence.

In a further aspect, the invention encompasses methods and kits useful for the identification of such antagonists are also encompassed by the present invention.

Such methods comprise the step of contacting a FPRL1 receptor with a composition comprising a biologically active SHAAGtide, or protein or peptide comprising a

5 SHAAGtide sequence, in the presence of a candidate antagonist molecule.

Antagonists to FPRL1 receptor function may be identified as those compounds reducing receptor activity compared to that observed in the absence of the candidate compound.

## 10 DESCRIPTION OF THE DRAWINGS

**Figure 1.** Table showing reported FPRL1 endogenous low affinity ligands and non-natural ligands.

**Figure 2.** Figure showing the amino acid sequence alignment of the human CCL23/CK $\beta$ 8 variants with human CCL15/MIP-1 $\alpha$  and CCL3/MIP-1 $\delta$ .

15

## DETAILED DESCRIPTION

The inventors have discovered that a CK $\beta$ 8-1 truncation variant, CK $\beta$ 8-1 (25-116), is a bifunctional ligand for two distinct GPCRs: the chemokine receptor CCR1 and the formyl peptide receptor like 1 receptor (FPRL1). The inventors have also

20 discovered that, in addition to its activity as a CCR1 ligand, CK $\beta$ 8-1(25-116) is involved in inflammatory reactions and immunity by recruiting monocytes and neutrophils through its role as a functional ligand for FPRL1. CK $\beta$ 8 attracts cells including monocytes, dendritic cells and resting lymphocytes through CCR1, but lacks the alternatively-spliced exon found in CK $\beta$ 8-1(25-116) (SHAAGtide

25 sequence). CK $\beta$ 8-1(1-116), the alternatively-spliced form of CK $\beta$ 8 (116 amino acids) is a functional ligand for the CCR1 receptor, as is CK $\beta$ 8. However, CK $\beta$ 8-1(1-116) does not exert its functions through the SHAAGtide sequence.

The inventors have also discovered a class of novel peptides (the SHAAGtide peptide and variants of the SHAAGtide peptide - henceforth collectively known as

30 "SHAAGtides"), truncation mutants of the splice exon of the CC chemokine CCL23, CK $\beta$ 8-1(25-116), that are surprisingly effective and valuable ligands for the FPRL1 receptor. These peptides produce a calcium flux in leukocytes expressing the FPRL1. In addition, SHAAGtides effectively attract cells including monocytes, neutrophils,

mature dendritic cells (mDCs) and immature dendritic cells (iDCs) and other leukocyte subsets. The SHAAGtide peptide (SEQ ID NO:1) and certain SHAAGtide variants, along with their parent chemokine CK $\beta$ 8-1 (25-116), are functional on both monocytes and neutrophils that are known to express FPRL1. Functional

5 SHAAGtides generate calcium flux upon receptor-ligand binding in leukocytes and attract monocytes, neutrophils, mature dendritic cells (mDCs), and immature dendritic cells (iDCs) in chemotactic assays. In light of these observations, the SHAAGtides represent cryptic functional peptides that are therefore surprisingly effective as FPRL1 ligands.

10 The invention encompasses SHAAGtides as well as proteins and peptides comprising SHAAGtides, with the exception of CK $\beta$ 8-1 (25-116) and CK $\beta$ 8-1 (1-116). In addition, the invention also includes nucleic acids encoding SHAAGtides, as well as nucleic acids encoding proteins and peptides comprising SHAAGtides, with the exception of nucleic acids encoding CK $\beta$ 8-1 (25-116) ) and CK $\beta$ 8-1 (1-116).

15 Compositions containing the SHAAGtides as well as proteins and peptides comprising SHAAGtides, including CK $\beta$ 8-1 (25-116) are also included in the invention. Such compositions include those suitable for administration to a subject to enhance FPRL1 activity. Also included are kits comprising such compositions. Such kits may be assembled to facilitate administration of, for example, pharmaceutical  
20 compositions.

The invention also encompasses methods of treating a subject in need of stimulation of inflammatory reactions and innate immunity. Stimulating such activity may benefit subjects suffering from diseases, for example, infectious diseases (and also in vaccination, as described in co-pending patent application "Methods and  
25 Compositions for Inducing an Immune Response", filed May 7<sup>th</sup>, 2002 – attorney reference number 10709/23). Such methods comprise stimulating the FPRL1 receptor by administering a composition comprising a SHAAGtide, a peptide or protein comprising a SHAAGtide, or other stimulatory molecule.

The invention also encompasses methods of treating a subject in need of a  
30 downregulation of inflammatory reactions and innate immunity. Downregulation of such activity may benefit subjects suffering from diseases including neurodegenerative disorders, such as Alzheimer's disease or Creutzfeldt-Jakob

disease. Such methods comprise downregulating the FPRL1 receptor by administering a composition comprising an antagonist to FPRL1 receptor function.

Methods and kits for the identification of such antagonists are also encompassed by the present invention. Such methods comprise the step of contacting  
5 a FPRL1 receptor with a composition comprising a biologically active SHAAGtide sequence, a peptide or protein comprising an active SHAAGtide, in the presence of a candidate antagonist molecule. Antagonists to FPRL1 receptor function may be identified as those compounds reducing receptor activity compared to that observed in  
10 the absence of the candidate compound. Such methods may be performed in vitro or in vivo. In addition, kits may be assembled to facilitate such in vitro or in vivo tests.

#### **SHAAGtides and molecules comprising SHAAGtides.**

##### *SHAAGtide peptides and polypeptides comprising SHAAGtides*

Table 1 shows the SHAAGtide polypeptide sequence (SEQ ID NO:1) and the  
15 polypeptide sequences of certain SHAAGtide truncated variants and other variants. Table 2 shows the SHAAGtide polynucleotide sequence (SEQ ID NO:12) and the polynucleotide sequences of SHAAGtide truncated variants and other variants. Table 3 shows the human CK $\beta$ 8-1(25-116) Nucleotide Sequence (SEQ ID NO:20). Figure 2 shows the amino acid sequence alignment of the human CCL23/CK $\beta$ 8 variants (CK $\beta$   
20 (1-99) - SEQ ID NO: 13; CK $\beta$  (25-99) - SEQ ID NO: 14; CK $\beta$  (1-116) - SEQ ID NO: 15; CK $\beta$  (25-116) - SEQ ID NO: 16) with human CCL15/MIP-1 $\alpha$  (SEQ ID NO: 19); CCL3/MIP-1 $\delta$  (SEQ ID NO: 17) and Leukotactin (SEQ ID NO: 18). Four conserved cysteine residues are shown in boxes and two additional cysteines, not normally found in the CC chemokine family, are shown in dashed boxes. The alternatively spliced  
25 exon of CCL23/CK $\beta$ 8-1 is shown underlined.

**Table 1** SHAAGtide and various truncated and other variants  
- amino acid sequences.

SEQ ID NO:	Designation and FPRL1 Activity	Amino acid sequence
1	CCXP1 Native sequence; high activity	Met Leu Trp Arg Arg Lys Ile Gly Pro Gln Met Thr Leu Ser His Ala 1 5 10 15 Ala Gly 18
2	CCXP2 Low activity	Arg Arg Lys Ile Gly Pro Gln Met Thr Leu Ser His Ala Ala Gly 1 5 10 15
3	CCXP3 High activity	Met Leu Trp Arg Arg Lys Ile Gly Pro Gln Met Thr Leu Ser His 1 5 10 15
4	CCXP4 Low activity	Ile Gly Pro Gln Met Thr Leu Ser His Ala Ala Gly 1 5 10
5	CCXP5 Moderate activity	Met Leu Trp Arg Arg Lys Ile Gly Pro Gln Met Thr 1 5 10
6	CCXP6 high activity	Met Leu Trp Arg Arg Lys Ile Gly Pro Gln Met Thr Leu Ser His Ala 1 5 10 15 Ala Tyr 18
7	CCXP7 Low activity	Trp Arg Arg Lys Ile Gly Pro Gln Met Thr Leu Ser His Ala Ala Gly 1 5 10 15
8	CCXP8 Moderate activity	Met Leu Trp Arg Arg Lys Ile Gly Pro Gln Met 1 5 10
9	CCXP9 Low activity	Trp Arg Arg Lys Ile Gly Pro Gln Met 1 5
10	CCXP10 Low activity	Trp Arg Arg Lys Ile Gly 1 5
11	CCXP11 Moderate activity	Leu Trp Arg Arg Lys Ile Gly Pro Gln Met Thr Leu Ser His 1 5 10

**Table 2** SHAAGtide and various truncated and other variants  
- polynucleotide sequences

SEQ ID NO:	Polynucleotide sequence
20	atgctctgga ggagaaagat tggtcctcag atgacccttt ctcatgctgc agga 54
21	aggagaaaga ttggtcctca gatgaccctt tctcatgctg cagga
22	atgctctgga ggagaaagat tggtcctcag atgacccttt ctcat 45
23	attggtcctc agatgaccct ttctcatgct gcagga
24	atgctctgga ggagaaagat tggtcctcag atgacc 36
25	atgctctgga ggagaaagat tggtcctcag atgacccttt ctcatgctgc atat 54
26	tggaggagaa agattggtcc tcagatgacc ctttctcatg ctgcagga
27	atgctctgga ggagaaagat tggtcctcag atg 33
28	tggaggagaa agattggtcc tcagatg
29	tggaggagaa agattggt
30	ctctggagga gaaagattgg tcctcagatg accctttctc at 42

5

**Table 3** Human CK $\beta$ 8-1(25-116) Nucleotide Sequence (SEQ ID NO:12)

<u>atgctctgga ggagaaagat tggtcctcag atgacccttt ctcatgctgc aggattccat</u>	60
gctactagtg ctgactgctg catctcctac accccacgaa gcatcccggtg ttactcctg	120
gagagttact ttgaaacgaa cagcgagtgc tccaagccgg gtgtcatctt cctcaccaag	180
aaggggcgac gtttctgtgc caacccagat gataagcaag ttcaggtttg catgagaatg	240
ctgaagctgg acacacggat caagaccagg aagaattga	279

*SHAAGtide molecules, derivatives and analogs*

10

SHAAGtide peptides of the present invention include those molecules listed in Table 1. In addition, various other derivatives of SHAAGtide peptides and nucleotides may be synthesized using standard techniques. Derivatives are nucleic acid sequences or amino acid sequences formed from native compounds either

directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar, but not identical, to the native compound but differ from it in respect to certain components or side chains. Analogs may be synthesized or from a different evolutionary origin.

5           Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid. For example, SEQ ID NO:3 contains only the first N-terminal 15 amino acids of the *SHAAGtide* molecule (SEQ ID NO:1). Derivatives or analogs of the *SHAAGtide* nucleic acid or peptide include, but are not limited to, molecules comprising regions that are  
10 substantially homologous to the *SHAAGtide* nucleic acid or peptide by at least about 70%, 80%, or 95% identity over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a homology algorithm, or whose encoding nucleic acid is capable of hybridizing to a complementary sequence encoding the aforementioned peptide sequences under  
15 stringent, moderately stringent, or low stringent conditions. (Ausubel et al., 1987.) A complementary nucleic acid molecule is one that is sufficiently complementary to a sequence, such that hydrogen bonds are formed with few mismatches, forming a stable duplex. "Complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides.

20           The specificity of single stranded DNA to hybridize complementary fragments is determined by the "stringency" of the reaction conditions. Hybridization stringency increases as the propensity to form DNA duplexes decreases. In nucleic acid hybridization reactions, the stringency can be chosen to either favor specific hybridizations (high stringency), which can be used to identify, for example, full-  
25 length clones from a library. Less-specific hybridizations (low stringency) can be used to identify related, but not exact, DNA molecules (homologous, but not identical) or segments.

          DNA duplexes are stabilized by: (1) the number of complementary base pairs, (2) the type of base pairs, (3) salt concentration (ionic strength) of the reaction  
30 mixture, (4) the temperature of the reaction, and (5) the presence of certain organic solvents, such as formamide which decreases DNA duplex stability. In general, the longer the probe, the higher the temperature required for proper annealing. A common approach is to vary the temperature: higher relative temperatures result in

more stringent reaction conditions. (Ausubel et al., 1987) provide an excellent explanation of stringency of hybridization reactions.

To hybridize under "stringent conditions" describes hybridization protocols in which nucleotide sequences at least 60% homologous to each other remain  
5 hybridized. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are  
10 generally present at excess, at  $T_m$ , 50% of the probes are occupied at equilibrium.

"Stringent hybridization conditions" conditions enable a probe, primer or oligonucleotide to hybridize only to its target sequence. Stringent conditions are sequence-dependent and will differ. Stringent conditions comprise: (1) low ionic strength and high temperature washes (e.g. 15 mM sodium chloride, 1.5 mM sodium  
15 citrate, 0.1 % sodium dodecyl sulfate at 50°C); (2) a denaturing agent during hybridization (e.g. 50% (v/v) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50mM sodium phosphate buffer (pH 6.5; 750 mM sodium chloride, 75 mM sodium citrate at 42°C); or (3) 50% formamide. Washes typically also comprise 5X SSC (0.75 M NaCl, 75 mM sodium citrate), 50 mM  
20 sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. Preferably, the conditions are such that sequences at least  
25 about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. These conditions are presented as examples and are not meant to be limiting.

"Moderately stringent conditions" use washing solutions and hybridization conditions that are less stringent (Sambrook, 1989), such that a polynucleotide will  
30 hybridize to the entire, fragments, derivatives or analogs of SEQ ID NOS:7-12, 14. One example comprises hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. The temperature, ionic strength, etc., can be

adjusted to accommodate experimental factors such as probe length. Other moderate stringency conditions have been described (Ausubel et al., 1987; Kriegler, 1990).

"Low stringent conditions" use washing solutions and hybridization conditions that are less stringent than those for moderate stringency (Sambrook, 1989), such that a polynucleotide will hybridize to the entire, fragments, derivatives or analogs of SEQ ID NOS:7-12, 14,. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency, such as those for cross-species hybridizations are well-described (Ausubel et al., 1987; Kriegler, 1990; Shilo and Weinberg, 1981).

In addition to naturally-occurring allelic variants of SHAAGtide, changes can be introduced by mutation into SEQ ID NO:1 that incur alterations in the amino acid sequences of the encoded SHAAGtide that do not significantly alter SHAAGtide function. For example, an amino acid substitution at the C-terminal amino acid residue has been made in the sequence of SEQ ID NO:6. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the SHAAGtide without altering biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the SHAAGtide of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well known in the art.

Useful conservative substitutions are shown in Table 4, "Preferred substitutions." Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the invention so long as the substitution does not materially alter the biological activity of the compound.

Table 4 Preferred substitutions

Original residue	Exemplary substitutions	Preferred substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys

Table 4 Preferred substitutions

Original residue	Exemplary substitutions	Preferred substitutions
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, Norleucine	Leu

Non-conservative substitutions that effect (1) the structure of the polypeptide backbone, such as a  $\beta$ -sheet or  $\alpha$ -helical conformation, (2) the charge, (3) hydrophobicity, or (4) the bulk of the side chain of the target site can modify

- 5 SHAAGtide function, especially when a SHAAGtide sequences comprises a part of a larger polypeptide molecule. Residues are divided into groups based on common side-chain properties as denoted in Table 5. Non-conservative substitutions entail exchanging a member of one of these classes for another class. Substitutions may be introduced into conservative substitution sites or more preferably into non-conserved
- 10 sites.

Table 5 Amino acid classes

Class	Amino acids
hydrophobic	Norleucine, Met, Ala, Val, Leu, Ile
neutral hydrophilic	Cys, Ser, Thr
acidic	Asp, Glu
basic	Asn, Gln, His, Lys, Arg
disrupt chain conformation	Gly, Pro
aromatic	Trp, Tyr, Phe

The variant polypeptides can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR  
5 mutagenesis. Site-directed mutagenesis (Carter, 1986; Zoller and Smith, 1987), cassette mutagenesis, restriction selection mutagenesis (Wells et al., 1985) or other known techniques can be performed on the cloned DNA to produce the *SHAAGtide* variant DNA (Ausubel et al., 1987; Sambrook, 1989).

An "isolated" or "purified" *SHAAGtides* of the present invention comprise  
10 polypeptides, proteins or biologically active fragments separated and/or recovered from a component of its natural environment. Isolated *SHAAGtides* include those expressed heterologously in genetically engineered cells or expressed *in vitro*.

Contaminant components include materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide. To be substantially isolated,  
15 preparations having less than 30% by dry weight of non-*SHAAGtide* contaminating material (contaminants), more preferably less than 20%, 10% and most preferably less than 5% contaminants.

Polypeptides and fragments of interest can be produced by any method well known in the art, such as by expression via vectors such as bacteria, viruses and  
20 eukaryotic cells. In addition, *in vitro* synthesis, such as peptide synthesis, may be also used.

An "active polypeptide or polypeptide fragment" retains a biological and/or an immunological activity similar, but not necessarily identical, to an activity of a *SHAAGtide* polypeptide shown in Table 1. Immunological activity, in the context of

this immediate discussion of the polypeptide *per se*, and not an actual biological role for SHAAGtide in eliciting or enhancing FPRL1 activity, refers to an aspect of a SHAAGtide polypeptide in that a specific antibody against a SHAAGtide antigenic epitope binds a SHAAGtide. Biological activity refers to a function, either inhibitory or stimulatory, caused by a native SHAAGtide polypeptide. A biological activity of SHAAGtide polypeptide includes, for example, binding to the FPRL1 receptor, or chemotaxis or eliciting calcium flux upon FPRL1 receptor binding. A particular biological assay (see Examples), with or without dose dependency, can be used to determine SHAAGtide activity. A nucleic acid fragment encoding a biologically-active portion of SHAAGtide can be prepared by isolating a polynucleotide sequence that encodes a polypeptide having a SHAAGtide biological activity, expressing the encoded portion of SHAAGtide (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of SHAAGtide polypeptide.

In general, a SHAAGtide polypeptide variant that preserves SHAAGtide polypeptide-like function and includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further includes the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

Table 1 shows that the deletion of amino acids at the C-terminal of the SHAAGtide sequence is less likely to cause a loss of FPRL1 activity than deletion at the N-terminal (see Example 9). For example, SEQ ID NO:8, consisting of the 11 N-terminal amino acids of the SHAAGtide sequence still retains moderate FPRL1 activity. However, deletion of 3 N-terminal amino acids (SEQ ID NO:2) results in only a low FPRL1 activity. Nevertheless, the deletion of the terminal amino acid at the N-terminal (SEQ ID NO:11) does not result in a complete loss in FPRL1 activity.

"SHAAGtide variant" means an active SHAAGtide polypeptide having at least: (1) about 80% amino acid sequence identity with a full-length native sequence SHAAGtide polypeptide sequence or (2) any fragment of a full-length SHAAGtide polypeptide sequence. For example, SHAAGtide polypeptide variants include SHAAGtide polypeptides wherein one or more amino acid residues are added or deleted at the N- or C- terminus of the full-length native amino acid sequence, with

the exception of those fragments that are identical to CK $\beta$ 8 and CK $\beta$ 8-1. A SHAAGtide polypeptide variant will have at least about 80% amino acid sequence identity, preferably at least about 81% amino acid sequence identity, more preferably at least about 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,  
5 94%, 95%, 96%, 97%, 98% amino acid sequence identity and most preferably at least about 99% amino acid sequence identity with a full-length native sequence SHAAGtide polypeptide sequence. Ordinarily, SHAAGtide variant polypeptides are at least about 10 amino acids in length, often at least about 20 amino acids in length, more often at least about 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or 300 amino acids  
10 in length, or more.

"Percent (%) amino acid sequence identity" is defined as the percentage of amino acid residues in SHAAGtide that are identical with amino acid residues in a candidate sequence when the two sequences are aligned. To determine % amino acid identity, sequences are aligned and if necessary, gaps are introduced to achieve the  
15 maximum % sequence identity; conservative substitutions are not considered as part of the sequence identity. Amino acid sequence alignment procedures to determine percent identity are well known to those of skill in the art. Often publicly available computer software such as BLAST, BLAST2, ALIGN2 or Megalign (DNASTAR) software is used to align peptide sequences.

20 When amino acid sequences are aligned, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) can be calculated as:

25 
$$\% \text{ amino acid sequence identity} = X/Y \cdot 100$$

where

X is the number of amino acid residues scored as identical matches by the sequence alignment program's or algorithm's alignment of A and B

and

30 Y is the total number of amino acid residues in B.

If the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

Fusion polypeptides are useful in expression studies, cell-localization, bioassays, and SHAAGtide purification. A SHAAGtide "chimeric protein" or "fusion protein" comprises SHAAGtide fused to a non-SHAAGtide polypeptide. A non-SHAAGtide polypeptide is not substantially homologous to a SHAAGtide polypeptide. A SHAAGtide fusion protein may include any portion to the entire SHAAGtide, including any number of the biologically active portions. For example, SHAAGtide may be fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins facilitate the purification of recombinant SHAAGtide. In certain host cells, (e.g. mammalian), heterologous signal sequences fusions may ameliorate SHAAGtide expression and/or secretion.

Antibodies specific to the SHAAGtide and SHAAGtide variant sequences are also encompassed by the invention. Methods of producing polyclonal and monoclonal antibodies, including binding fragments (e.g.,  $F_{(ab)2}$ ) and single chain versions are well known. Hence, polyclonal or monoclonal antibodies can be prepared by standard techniques.

The chemotactic compositions of the invention contain one or more polynucleotides or polypeptides containing a SHAAGtide sequence. In an embodiment, the composition contains a SHAAGtide that is an isolated or recombinant polynucleotide or polypeptide. In an embodiment, the SHAAGtide(s) is/are the predominant species (i.e., greater than about 50%, more often greater than about 80% by weight of the total of the members of the class of molecule in the composition) of its class (e.g., polypeptide, polynucleotide, lipid, carbohydrate) in the composition. The chemotactic compositions of the invention contain SHAAGtides free of materials normally associated with their *in situ* environment (if naturally occurring).

An isolated SHAAGtide nucleic acid molecule is purified from the setting in which it is found in nature and is separated from at least one contaminant nucleic acid molecule. Isolated SHAAGtide molecules are distinguished from the specific SHAAGtide molecule, as it exists in cells.

#### **Use of SHAAGtide compositions in the treatment of disease.**

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder

associated with aberrant FPRL1 receptor or FPRL1 ligand activity. Examples include neurodegenerative disorders, such as Alzheimer's Disease.

Diseases or conditions of humans or other species which can be treated with SHAAGtides or proteins or peptides comprising SHAAGtides, or inhibitors or agonists of FPLR1-SHAAGtide interactions, include, but are not limited to, peripheral  
5 - chronic inflammation-related diseases, for example: chronic inflammation; thrombosis; atherosclerosis; restenosis; chronic venous insufficiency; recurrent bacterial infections; sepsis; cutaneous infections; renal disease; glomerulonephritis; fibrotic lung disease; allergic disease; IBS; rheumatoid arthritis and acute  
10 bronchiolitis. Central nervous system - macroglia and microglia related diseases, for example: neurodegenerative diseases; Alzheimer's disease; Multiple sclerosis; Parkinson's disease; neuroinflammation; HIV-associated neurological diseases; HIV-associated dementia; CNS bacterial infections; brain *Toxoplasma gondii*; *Acanthamoeba* infections; *Listeria* infections; prion diseases; subacute spongiform  
15 encephalopathies and macular degeneration may also be treated.

Diseases and disorders that are characterized by increased FPRL1 levels or biological activity may be treated with therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Antagonists may be administered in a therapeutic or prophylactic manner. Therapeutics that may be used include: (1) molecules comprising inactive  
20 SHAAGtide peptides, or analogs, derivatives, fragments or homologs thereof; (2) *SHAAGtide* antisense nucleic acids (3) antibodies to SHAAGtide peptides, or analogs, derivatives, fragments or homologs thereof or (4) modulators (*i.e.*, inhibitors and antagonists) that antagonize the activity of the FPRL1 receptor.

Diseases and disorders that are characterized by decreased FPRL1 levels or  
25 biological activity may be treated with therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that up regulate activity may be administered therapeutically or prophylactically. Therapeutics that may be used include peptides, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability. Therapeutics that may be used include: (1) molecules comprising  
30 SHAAGtide peptides, or analogs, derivatives, fragments or homologs thereof; (2) *SHAAGtide* nucleic acids; or (3) modulators that agonize the activity of the FPRL1 receptor.

The invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant FPRL1 receptor expression or activity, by

administering an agent that modulates a FPRL1 activity. Subjects at risk for a disease that is caused or contributed to by aberrant FPRL1 activity can be identified by, for example, any or a combination of diagnostic or prognostic assays. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FPRL1 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of FPRL1 aberrancy, for example, a FPRL1 agonist or FPRL1 antagonist can be used to treat the subject. The appropriate agent can be determined based on screening assays.

Another aspect of the invention pertains to methods of modulating FPRL1 activity for therapeutic purposes. Modulatory methods involve contacting a cell with an agent that modulates one or more of the activities of FPRL1 activity associated with the cell. An agent that modulates FPRL1 activity can be a nucleic acid or a protein, a naturally occurring cognate ligand of FPRL1, a peptide, a SHAAGtide peptidomimetic, or other small molecule. The agent may stimulate FPRL1 activity. The agent may inhibit a FPRL1 activity. Modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). For example, the method may involve administering a SHAAGtide or nucleic acid molecule as therapy to compensate for reduced or aberrant FPRL1 or FPRL1 ligand expression or activity.

Stimulation of FPRL1 activity is desirable in situations in which FPRL1, or FPRL1 ligand is abnormally down-regulated and/or in which increased FPRL1, or FPRL1 ligand activity is likely to have a beneficial effect; for example, in treating an infection or in vaccination. Conversely, diminished FPRL1, or FPRL1 ligand activity is desired in conditions in which FPRL1, or FPRL1 ligand activity is abnormally up-regulated and/or in which decreased FPRL1, or FPRL1 ligand activity is likely to have a beneficial effect; for example, in treating chronic inflammation.

Suitable *in vitro* or *in vivo* assays can be performed to determine the effect of a specific therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given therapeutic exerts the desired effect upon the cell type(s). Modalities for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, dogs and the like, prior to testing in

human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Diseases and conditions associated with inflammation and infection can be treated using the methods of the present invention. The disease or condition is one in which the actions of a FPRL1 ligand on a FPRL1 receptor is to be inhibited or promoted, in order to modulate the immune response.

The compositions of the present invention may be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes of administration and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration. In addition to the treatment of warm-blooded animals such as mice, rats, horses, cattle, sheep, dogs, cats, monkeys, etc., the compositions of the invention are effective for use in humans.

Combined therapy to modulate FPLR1 or FPLR1 ligand activity and thereby prevent and treat infectious diseases or inflammatory disorders and diseases is illustrated by the combination of the compounds of this invention and other compounds which are known for such utilities.

For example, in the treatment or prevention of inflammation, the present compounds may be used in conjunction with an anti-inflammatory or analgesic agent such as an opiate agonist, a lipooxygenase inhibitor, such as an inhibitor of 5-lipoxygenase, a cyclooxygenase inhibitor, such as a cyclooxygenase-2 inhibitor, an interleukin inhibitor, such as TNF $\alpha$ , an interleukin-1 inhibitor, an NMDA antagonist, an inhibitor of nitric oxide or an inhibitor of the synthesis of nitric oxide, a non-steroidal anti-inflammatory agent, or a cytokine-suppressing anti-inflammatory agent, for example with a compound such as acetaminophen, aspirin, codeine, fentanyl, ibuprofen, indomethacin, ketorolac, morphine, naproxen, phenacetin, piroxicam, a steroidal analgesic, sufentanil, sunlindac, tenidap, and the like. Similarly, the instant compounds may be administered with a pain reliever; a potentiator such as caffeine, an H2-antagonist, simethicone, aluminum or magnesium hydroxide; a decongestant such as phenylephrine, phenylpropanolamine, pseudophedrine, oxymetazoline, epinephrine, naphazoline, xylometazoline, propylhexedrine, or levo-desoxyephedrine; an anti-tussive such as codeine, hydrocodone, caramiphen,

carbetapentane, or dexamethorphan; a steroid; cyclosporin A; methotrexate; IL-10; a diuretic; and a sedating or non-sedating antihistamine.

*Pharmaceutical compositions*

5           Agonists or antagonists of the FPRL1 receptor can be incorporated into pharmaceutical compositions. Such compositions typically comprise the agonists or antagonists and a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the  
10 like, compatible with pharmaceutical administration (Gennaro (2000)). Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Except when a conventional media or agent is incompatible with an active compound, use of these  
15 compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the agonist or antagonist is formulated to be compatible with its intended route of administration, including intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical),  
20 transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating  
25 agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

30           Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline,

bacteriostatic water, CREMOPHOR EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid so as to be administered using a syringe. Such compositions should be stable during manufacture and storage and must be preserved against contamination from microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (such as glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures. Proper fluidity can be maintained, for example, by using a coating such as lecithin, by maintaining the required particle size in the case of dispersion and by using surfactants. Various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, and thimerosal, can contain microorganism contamination. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride can be included in the composition. Compositions that can delay absorption include agents such as aluminum monostearate and gelatin.

15 Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients as required, followed by sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium, and the other required ingredients. Sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying that yield a powder containing the active ingredient and any desired ingredient from a sterile solutions.

25 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included. Tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, PRIMOGEL, or corn starch; a lubricant such as magnesium stearate or STEROTES; a glidant such as

colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered as an aerosol spray from a nebulizer or a pressurized container that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide.

Systemic administration can also be transmucosal or transdermal. For transmucosal or transdermal administration, penetrants that can permeate the target barrier(s) are selected. Transmucosal penetrants include, detergents, bile salts, and fusidic acid derivatives. Nasal sprays or suppositories can be used for transmucosal administration. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams.

The compounds can also be prepared in the form of suppositories (*e.g.*, with bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable or biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Polyethylene glycols, *e.g.* PEG, are also good carriers. Such materials can be obtained commercially from ALZA Corporation (Mountain View, CA) and NOVA Pharmaceuticals, Inc. (Lake Elsinore, CA), or prepared by one of skill in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, such as in (Eppstein et al. US Patent No. 4,522,811. 1985).

Oral formulations or parenteral compositions in unit dosage form can be created to facilitate administration and dosage uniformity. Unit dosage form refers to physically discrete units suited as single dosages for the subject to be treated, containing a therapeutically effective quantity of active compound in association with the required pharmaceutical carrier. The specification for the unit dosage forms of the invention are dictated by, and directly dependent on, the unique characteristics of the active compound and the particular desired therapeutic effect, and the inherent limitations of compounding the active compound.

The nucleic acid molecules of *SHAAGtide* can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (Nabel and Nabel, US Patent No. 5,328,470, 1994), or by stereotactic injection (Chen et al. (1994)). The

5 pharmaceutical preparation of a gene therapy vector can include an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

10 In one aspect, the *SHAAGtide* is delivered as DNA such that the polypeptides are generated *in situ*. In one embodiment, the DNA is "naked," as described, for example, in Ulmer *et al.* (1993) and reviewed by Cohen, (1993). The uptake of naked DNA may be increased by coating the DNA onto a carrier, e.g. biodegradable beads, which is efficiently transported into the cells. In such vaccines, the DNA may be  
15 present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems.

Vectors, used to shuttle genetic material from organism to organism, can be divided into two general classes: Cloning vectors are replicating plasmid or phage  
20 with regions that are non-essential for propagation in an appropriate host cell and into which foreign DNA can be inserted; the foreign DNA is replicated and propagated as if it were a component of the vector. An expression vector (such as a plasmid, yeast, or animal virus genome) is used to introduce foreign genetic material into a host cell or tissue in order to transcribe and translate the foreign DNA, such as *SHAAGtide*. In  
25 expression vectors, the introduced DNA is operably-linked to elements such as promoters that signal to the host cell to transcribe the inserted DNA. Some promoters are exceptionally useful, such as inducible promoters that control gene transcription in response to specific factors. Operably-linking a *SHAAGtide* polynucleotide to an inducible promoter can control the expression of a *SHAAGtide* polypeptide or  
30 fragments. Examples of classic inducible promoters include those that are responsive to  $\alpha$ -interferon, heat shock, heavy metal ions, and steroids such as glucocorticoids (Kaufman, 1990. *Methods Enzymol* 185: 487-511.) and tetracycline. Other desirable inducible promoters include those that are not endogenous to the cells in which the construct is being introduced, but, however, are responsive in those cells when the

induction agent is exogenously supplied. In general, useful expression vectors are often plasmids. However, other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses) are contemplated.

5        Vector choice is dictated by the organism or cells being used and the desired fate of the vector. Vectors may replicate once in the target cells, or may be "suicide" vectors. In general, vectors comprise signal sequences, origins of replication, marker genes, enhancer elements, promoters, and transcription termination sequences.

10        The pharmaceutical composition may further comprise other therapeutically active compounds as noted herein which are usually applied in the treatment of FPRL1-related conditions.

      In the treatment or prevention of conditions which require FPRL1 modulation an appropriate dosage level of an agonist or antagonist will generally be about 0.01 to 500 mg per kg patient body weight per day which can be administered in single or  
15        multiple doses. Preferably, the dosage level will be about 0.1 to about 250 mg/kg per day; more preferably about 0.5 to about 100 mg/kg per day. A suitable dosage level may be about 0.01 to 250 mg/kg per day, about 0.05 to 100 mg/kg per day, or about 0.1 to 50 mg/kg per day. Within this range the dosage may be 0.05 to 0.5, 0.5 to 5 or 5 to 50 mg/kg per day. For oral administration, the compositions are preferably  
20        provided in the form of tablets containing 1.0 to 1000 milligrams of the active ingredient, particularly 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0, 75.0, 100.0, 150.0, 200.0, 250.0, 300.0, 400.0, 500.0, 600.0, 750.0, 800.0, 900.0, and 1000.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. The compounds may be administered on a regimen of 1 to 4 times per day,  
25        preferably once or twice per day.

      However, the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of  
30        administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

*Kits*

In an aspect, the invention provides kits containing one or more of the following in a package or container: (1) a biologically active composition of the invention or an FPRL1 antagonist; (2) a pharmaceutically acceptable adjuvant or excipient; (3) a vehicle for administration, such as a syringe; (4) instructions for administration. Embodiments in which two or more of components (1) - (4) are found in the same container are also contemplated.

When a kit is supplied, the different components of the composition may be packaged in separate containers and admixed immediately before use. Such packaging of the components separately may permit long-term storage without losing the active components' functions.

The reagents included in the kits can be supplied in containers of any sort such that the life of the different components are preserved and are not adsorbed or altered by the materials of the container. For example, sealed glass ampules may contain lyophilized SHAAGtide polypeptide or polynucleotide, or buffers that have been packaged under a neutral, non-reacting gas, such as nitrogen. Ampules may consist of any suitable material, such as glass, organic polymers, such as polycarbonate, polystyrene, *etc.*; ceramic, metal or any other material typically employed to hold similar reagents. Other examples of suitable containers include simple bottles that may be fabricated from similar substances as ampules, and envelopes, that may comprise foil-lined interiors, such as aluminum or an alloy. Other containers include test tubes, vials, flasks, bottles, syringes, or the like. Containers may have a sterile access port, such as a bottle having a stopper that can be pierced by a hypodermic injection needle. Other containers may have two compartments that are separated by a readily removable membrane that upon removal permits the components to be mixed. Removable membranes may be glass, plastic, rubber, *etc.*

Kits may also be supplied with instructional materials. Instructions may be printed on paper or other substrate, and/or may be supplied as an electronic-readable medium, such as a floppy disc, CD-ROM, DVD-ROM, Zip disc, videotape, audiotape, *etc.* Detailed instructions may not be physically associated with the kit; instead, a user may be directed to an internet web site specified by the manufacturer or distributor of the kit, or supplied as electronic mail.

**Screening and detection methods**

SHAAGtides (and SHAAGtide nucleotides used to express SHAAGtides) can be used as reagents in methods to screen for compounds that modulate FPRL1  
5 receptor activity. Such compounds may be useful in treating disorders characterized by insufficient or excessive production of FPRL1 receptor or FPRL1 receptor ligand, or production of FPRL1 receptor or FPRL1 receptor ligand forms that have aberrant activity compared to wild-type molecules. In general, such compounds may be used to modulate biological functions that involve FPRL1 receptor/ FPRL1 receptor ligand.

10 The invention provides methods (screening assays) for identifying modalities, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs), foods, combinations thereof, *etc.*, that affect the FPRL1 receptor or FPRL1 receptor ligand. This may be a stimulatory or inhibitory effect. The invention also includes compounds identified in such screening assays.

15 Testing for compounds that increase or decrease FPRL1 receptor activity in response to or independent of a ligand is desirable. A compound may modulate FPRL1 receptor activity by increasing or decreasing the activity of FPRL1 receptor itself (agonists and antagonists).

Test compounds can be obtained using any of the numerous approaches in  
20 combinatorial library methods, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptides, while the other four approaches encompass peptide, non-peptide  
25 oligomer or small molecule libraries of compounds (Lam, 1997).

A "small molecule" refers to a composition that has a molecular weight of less than about 5 kD and more preferably less than about 4 kD, and most preferably less than 0.6 kD. Small molecules can be, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules.  
30 Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention. Examples of methods for the synthesis of molecular libraries have been described (Carell et al., 1994a; Carell et al., 1994b; Cho et al., 1993; DeWitt et al., 1993; Gallop et al., 1994; Zuckermann et al., 1994).

Libraries of compounds may be presented in solution (Houghten et al., 1992) or on beads (Lam et al., 1991), on chips (Fodor et al., 1993), bacteria, spores (Ladner et al., US Patent No. 5,223,409, 1993), plasmids (Cull et al., 1992) or on phage (Cwirla et al., 1990; Devlin et al., 1990; Felici et al., 1991; Ladner et al., US Patent  
5 No. 5,223,409, 1993; Scott and Smith, 1990).

Many assays for screening candidate or test compounds that bind to or modulate the activity of the FPRL1 receptor are available. A cell-free assay comprises, for example, contacting the FPRL1 receptor or biologically-active fragment with a SHAAGtide compound that binds the FPRL1 receptor to form an  
10 assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the FPRL1 receptor, where determining the ability of the test compound to interact with the FPRL1 receptor comprises determining the ability of the FPRL1 receptor to preferentially bind to or modulate the activity of the test compound. Cell-based assays include, for example,  
15 the calcium flux assays, binding assays and cellular migration assays discussed in the examples.

Immobilizing either a molecule containing a SHAAGtide sequence or one of its partner molecules (such as FPRL1) can facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate high  
20 throughput assays. Binding of a test compound to a SHAAGtide molecule or a FPRL1 receptor molecule, or interaction of SHAAGtide molecule with a FPRL1 receptor molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants, such as microtiter plates, test tubes, and micro-centrifuge tubes. A fusion protein can be provided that  
25 adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST (glutathione S-transferase)-SHAAGtide fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (SIGMA Chemical, St. Louis, MO) or glutathione derivatized microtiter plates that are then combined with the test compound or the test compound and either the non-adsorbed FPRL1  
30 receptor or SHAAGtide molecule, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly. Alternatively, the complexes can be

dissociated from the matrix, and the level of SHAAGtide binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in screening assays. See, for example co-pending United States Patent Application  
5 Serial Number 09/721, 902. Either a SHAAGtide molecule or a FPRL1 receptor molecule can be immobilized using biotin-avidin or biotin-streptavidin systems. Biotinylation can be accomplished using many reagents, such as biotin-NHS (N-hydroxy-succinimide; PIERCE Chemicals, Rockford, IL), and immobilized in wells of streptavidin-coated 96 well plates (PIERCE Chemical). Alternatively,  
10 antibodies or antibody fragments reactive with SHAAGtide molecules or FPRL1 receptor molecules but which do not interfere with binding of the SHAAGtide to the FPRL1 receptor molecule can be derivatized to the wells of the plate, and FPRL1 receptor molecule or SHAAGtide trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described for the  
15 GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with FPRL1 receptor molecules or SHAAGtide molecules, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FPRL1 receptor molecules or SHAAGtide molecules.

To demonstrate that the compounds are antagonists of the FPRL1 receptor,  
20 one can determine if they inhibit the activity of a SHAAGtide on the receptor. Preferably such compounds have the at least one of the following characteristics:

- (1) potently inhibit binding of a SHAAGtide or a molecule comprising a SHAAGtide sequence to the FPRL1 receptor;
- (2) significant inhibition of the  $\text{Ca}^{2+}$  response of a SHAAGtide or a molecule  
25 comprising a SHAAGtide binding to FPRL1;
- (3) limited non-specific  $\text{Ca}^{2+}$  response; or
- (4) inhibition of chemotactic activity.

Standard *in vitro* binding assays may be employed to demonstrate the affinity of the compounds for the FPRL1 receptor (thereby inhibiting the activity of a  
30 SHAAGtide by competitive interaction with the receptor). See examples below. Preferably, the active compounds exhibit an  $\text{IC}_{50}$  value of  $<10 \mu\text{M}$ , more preferably  $<5 \mu\text{M}$ , most preferably  $<1 \mu\text{M}$ .

Compounds that inhibit the activity of SHAAGtide affect intracellular  $\text{Ca}^{2+}$  concentrations in SHAAGtide stimulated cells. Ligand binding to the FPRL1 receptor results in G-protein induced activation of phospholipase C, which leads to the conversion of phosphatidyl inositol phosphate into inositol phosphate and diacylglycerol. Inositol phosphate in turn binds to a receptor located at intracellular sites to release  $\text{Ca}^{2+}$  into the cytoplasm. In addition to  $\text{Ca}^{2+}$  concentration increases due to release from intracellular stores, binding of inositol phosphate to its receptor leads to an increased flux of extracellular calcium across the membrane and into the cell. Other G-protein signaling pathways may be involved.

Thus, the activation of the FPRL1 receptor by a SHAAGtide, and, subsequently, inhibition of the activation by the compounds of the invention can be determined by assaying for an increase in free intracellular  $\text{Ca}^{2+}$  levels. Typically, this can be achieved by the use of calcium-sensitive fluorescent probes such as quin-2, fura-2 and indo-1. The affect of the active compounds to block the  $\text{Ca}^{2+}$  response depends on the amount of active compound and chemokine present. Generally, when 10 nM of chemokine is present, 10  $\mu\text{M}$  of active compound should produce 20 to 100% inhibition of the  $\text{Ca}^{2+}$  response.

To determine whether the active compound produces a non-specific  $\text{Ca}^{2+}$  response, cells bearing multiple receptors, including the receptor to which the active compound is targeted, are incubated with compound. Cells are then stimulated with a ligand to the target receptor and sequentially followed by stimulation with ligands to other receptors found on the sample cells. A comparable response of non-target receptors to ligand in the presence or absence of compound indicates that the active compound is specific for the target receptor.

To determine chemotaxis, any cell migration assay format may be used, such as the ChemoTx<sup>®</sup> system (NeuroProbe, Rockville, MD) or any other suitable device or system (Bacon et al., 1988; Penfold et al., 1999). In brief, these cell migration assays work as follows. After harvesting and preparing the cells bearing the active target chemokine receptor, the cells are mixed with candidate antagonists. The mixture is placed into the upper chamber of the cell migration apparatus. To the lower chamber, a stimulatory concentration of chemokine ligand is added. The migration assay is then executed, terminated, and cell migration assessed.

The inventors have shown SHAAGtide activity on the FPRL1 receptor expressed on monocytes, neutrophils, Immature Dendritic Cells and Mature Dendritic Cells. Hence, such cells may be used in *in vitro* assay methods. Enriched or substantially purified cell populations can be used in *in vitro* chemotaxis assays.

5 These cell populations can be prepared by a variety of methods known in the art depending on the specific cell-type desired. Typically, substantially purified cell populations are prepared by culture under specific conditions, by physical characteristics such as behavior in a density gradient, by sorting according to characteristic markers (*e.g.*, by fluorescence activated cell sorting (FACS) using  
10 antibodies (preferably monoclonal antibodies) to cell-surface proteins, immunoprecipitation), or other methods.

Cells can be identified by histology (see, *e.g.*, Luna, 1968), by immunological staining and similar methods (see, *e.g.*, Harlow et al. 1998; Coligan *et al.*, 1991. Methods for preparing substantially purified cell compositions for use in *in vitro*  
15 chemotaxis assays are briefly described *infra* and in the Examples. However, the invention does not require that any particular purification method be used, so long as the desired cells are obtained; many variations and alternative methods are known to those of skill in the art. Further, many other purification and detection methods, including methods suitable for cells not specifically listed herein, are known in the art  
20 or can be easily developed. Further, cloned cell lines derived from immune system tissues can be used in the chemotaxis assays described herein, if desired. General immunological, purification and cell culture methods are described in Coligan *et al.* (1991), including supplements through 1999. Unless otherwise specified, cells in culture are incubated at 37°C in 5% CO<sub>2</sub>.

25 Suitable methods for monocyte purification are found in Bender *et al.*, 1996. (also see U.S. Pat. No. 5,994,126). Briefly, monocytes are isolated from PBMC by depleting T cells using immobilized antibodies against a pan T cell surface marker CD2. Conveniently, a commercially available source of CD2 antibodies attached to magnetic beads (Dyna; Lake Success, NY) is used. PBMC isolated from a buffy coat  
30 (typically 35 mls containing 400 x 10<sup>6</sup> PMBC) by conventional Ficoll gradient centrifugation methods are resuspended in MACS buffer (DPBS (HyClone; Logan, UT) with 1% BSA (Sigma)) at 20 x 10<sup>6</sup> cells per ml. DPBS is Dulbecco's Phosphate Buffered Saline (CaCl<sub>2</sub> (0.1 g/l), KCl (0.2 g/l), KH<sub>2</sub>PO<sub>4</sub> (0.2 g/l), MgCl<sub>2</sub>·6H<sub>2</sub>O (0.1 g/l), NaCl (8.0 g/l), Na<sub>2</sub>HPO<sub>4</sub> (2.16 g/l)). An appropriate amount of immobilized CD2 +

magnetic beads (typically 10  $\mu$ l per  $10^6$  cells) are added to the cells. The mixture is incubated for 15 minutes at 4°C with gentle rotation. The magnetically tagged T cells are removed from the unlabeled cells on a magnetic cell sorter (Dyna) according to the manufacturer's protocols. The unlabeled cells contain primarily monocytes and B cells.

B cells in the above preparation are removed by taking advantage of differential adhesion properties. Briefly, PBMC depleted of T cells are allowed to adhere to the plastic of a T-175 tissue culture flask ( $100 \times 10^6$  cells/ flask; Costar; Acton, MA) for 3 hours at 37°C. Non-adherent cells (comprising largely B cells) are aspirated. To completely remove non-adherent cells, the flasks are rinsed 3 more times with DPBS. The resulting cells are largely enriched (*i.e.*, > 90%) for monocytes.

Monocytes can also be isolated by positive selection of CD14 antigen. Briefly, PBMC isolated from peripheral blood, such as a buffy coat, by standard Ficoll gradient centrifugation methods are resuspended in MACS buffer at  $1 \times 10^6$  cells/ml. Immobilized antibodies against the CD14 surface antigen, such as CD14+ magnetic microbeads (Miltenyi) are added (1  $\mu$ l of beads per  $1 \times 10^6$  cells) and the mixture is incubated at 4°C for 15 minutes. Monocytes are separated from the other cell populations by passing the mixture through a positive selection column on a magnetic cell sorter (Miltenyi Biotech; Auburn, CA) according to manufacturers protocol. Monocytes that are retained on the column are eluted with MACS buffer after the column is removed from the MACS apparatus. Cells are then pelleted by centrifugation and resuspended in RPMI plus 10% FCS media at  $10^6$  cells per ml. Monocytes isolated by this method are cultured essentially the same way as those isolated by the CD2+ depletion method.

Suitable methods for purification of dendritic cells, including separate mature and immature populations, are known in the art. Substantially purified dendritic cells (including subpopulations of mature or immature cells) can be prepared by selective *in vitro* culture conditions.

Dendritic cells are widely distributed in all tissues that have contact with potential pathogens (*e.g.*, skin, gastrointestinal and respiratory tracts, and T cell-rich areas of the secondary lymphoid tissues). In the skin and upper respiratory tract they form a lattice of highly arborised cells (called Langerhans cells in the skin). After capturing antigen, dendritic cells in the peripheral tissues such as the skin and gut,

traffic via the draining lymphatics to the T cell areas of lymph nodes where they present the internalized antigen. Immature dendritic cells function to take up and process antigens. During subsequent migration to the draining lymph node, the DC matures. The mature dendritic cells functions as the key APC to initiate immune responses by inducing the proliferation of pathogen specific cytotoxic and helper T cells.

Substantially pure populations of dendritic cells can be produced by *in vitro* culture, *infra*). In addition, there are marked changes in expression of chemokine receptors during dendritic cell maturation which can be used to identify cell stage (Campbell *et al.* 1998; Chan *et al.* 1999; Dieu *et al.* 1998; Kellermann *et al.* 1999). For example, immature dendritic cells express predominately CCR1, CCR5, and CXCR4. Upon maturation, these receptors, with the exception of CXCR4, are down regulated.

In culture, immature forms of dendritic cells undergo maturation thought to be analogous to the events during migration of dendritic cells from the point of antigen contact until to the secondary lymphoid tissues. Human or macaque dendritic cells of various developmental stages can be generated in culture, from CD14<sup>+</sup> blood progenitors using specific cytokines. A separate lineage of dendritic cells can be differentiated from CD34<sup>+</sup> precursor cells from cord blood or bone marrow. In one embodiment of the invention, subpopulations of dendritic cells are generated for *in vitro* assays for identification of chemotactic compositions (*i.e.* to assess chemotaxin potency and selectivity against defined DC sub-types). Exemplary subpopulations of dendritic cells are: (1) immature peripheral blood monocyte derived cells; (2) mature peripheral blood monocyte derived cells, and (3) cells derived from CD34<sup>+</sup> precursors. Subpopulations are isolated or produced by a variety of methods known in the art. For example, immature and mature dendritic cells from PBMCs are produced according to Bender *et al. supra*.

Briefly, PBMCs are depleted of T cells using immobilized antibodies against the cell surface marker CD2 (present on all T cells). Commercially available CD2<sup>+</sup> dynabeads (Dynal) can be used according to manufacturer's protocol. The T-cell depleted mixture is separated into adherent versus non-adherent fractions by incubating the cells on tissue culture grade plastic for 3 hours at 37°C. Non-adherent cells are gently removed, and adherent cells (generally CD14<sup>+</sup> monocytes) are placed in culture media (*e.g.*, RPMI + 10% FCS) supplemented with 1000 U/mL each of

GM-CSF and IL-4 (R&D Systems, Minneapolis, MN) ("Day 1"). Between days 3-7 the cells begin to display a veiled morphology, and cytokines are replenished on days 2, 4, and 6, at which time the cells can be harvested as immature dendritic cells. In one embodiment, cells of this *in vitro* stage are isolated and used in the assay.

- 5 Approximately  $10 \times 10^6$  dendritic cells are typically obtained from  $400 \times 10^6$  PBMCs.

Day 7 immature dendritic cells exhibit typical dendritic cell morphology, with elongated cell body and many processes. The size of the cells increase significantly compared to the precursor monocytes. Immature dendritic cells can be characterized phenotypically by monitoring their expression of cell surface markers.

- 10 Immature dendritic cells (generated from peripheral blood monocytes or from bone marrow derived CD34+ precursors) can be further activated and differentiated to become mature dendritic cells. Two methods are primarily used: MCM (macrophage conditioned medium) and double-stranded RNA-poly (I:C) stimulation (Cella et al, 1999; Verdijk *et al.* 1999).

- 15 In the MCM method, day 6 immature dendritic cells are harvested by centrifugation and resuspended in at  $10^6$  cells/ml in maturation medium (*e.g.*, MCM diluted (up to 1:1 with RPMI containing 10% FCS). GM-CSF (1000 U/ml) and IL-4 (1000 U/ml) are added. Cells are cultured for three more days, without further addition of GM-CSF (1000 U/ml) and IL-4. Day 9 cells are used as mature dendritic  
20 cells.

- In the poly (I:C) method, day 6 immature dendritic cells are harvested and resuspended in the standard culture medium (RPMI plus 10% FCS) supplemented with 20  $\mu$ g/ml of poly (I:C) (Sigma), 1000 U/ml of GM-CSF and IL-4. Cells are cultured for another three days without additional cytokines. Day 9 cells are used as  
25 mature dendritic cells.

Mature dendritic cells generated by these two different methods exhibit phenotypic and functional properties distinct from those of immature dendritic cells or the precursor monocytes. Mature dendritic cells from each preparation are thoroughly characterized by FACS to ensure that the desirable cell types are obtained.

- 30 Notably, generated mature dendritic cells express significantly higher level of MHC class II on the cell surface than immature cells. Expression of CD80, CD83 and CD86 are also up-regulated. Chemokine receptor expression also changes dramatically during the maturation process. For instance, CCR1, CCR5 are down-regulated sharply in mature cells, while CCR7 is up-regulated and appears on the cell

surface within a few hours after addition of MCM. Functionally, mature dendritic cells are no longer capable of efficiently taking up antigen, but gain the ability to stimulate the proliferation of naïve T cells and B cells. Mature dendritic cells also change their migratory behaviors; they no longer respond to ligands for CCR1, CCR2 and CCR5, such as MIP-1 $\alpha$ , RANTES and MIP-1 $\beta$ . Instead, they respond to CCR7 ligands SLC and ELC.

MCM is prepared by as described by Romani *et al.* 1996, with minor modifications. Briefly, petri dishes (100 mm, Falcon) are coated with 5 ml of human Ig (10 mg/mL) for 30 min at 37°C and washed with PBS 2-3 times immediately before use. 50 x10<sup>6</sup> PBMC in 8 ml are layered onto human Ig-coated plates for 1-2 hours. Non-adherent cells are washed away and discarded. The adherent cells are incubated in fresh complete medium (RPMI + 10% normal human serum) at 37°C, and the resulting media (MCM) is collected after 24 hours. The TNF- $\alpha$  concentration in the MCM is determined by the standard ELISA method (*e.g.*, using a TNF- $\alpha$  ELISA kit (R&D Systems, Minneapolis, MN)). The final TNF- $\alpha$  level in MCM is adjusted to 50 U/ml by mixing an appropriate amount of MCM with RPMI/10% fetal calf serum.

Suitable methods for neutrophil purification are known in the art. According to one suitable method, whole fresh blood (WB) is diluted 1:1 with 3% dextran in a 50 ml centrifuge tube and allowed to sediment for 30 - 45 minutes at room temperature. Twenty-five ml of WB plus 25 ml dextran results in approximately 35 ml of supernatant after 30 minutes sedimentation. The supernatant is layered over 12-15 ml Ficoll and centrifuged at 400 x g for 30-40 minutes at 18-20°C. The plasma/platelet layer containing mononuclear cells and Ficoll-Paque are removed by aspiration. Neutrophils are found in the white layer above the erythrocyte (RBC) layer. (In some preparations, the neutrophil and erythrocyte layers are mixed. In these cases, RBCs are removed by hypotonic lysis: 12.5 ml of cold 0.2% NaCl is added to the neutrophils/RBC pellet while vortexing. 12.5 ml of cold 1.6% NaCl is immediately added while still vortexing. The cells are centrifuged at 60- 100 x g for 10 m and recovered. If necessary the lysis step is repeated). The resulting neutrophils are >95% pure (with the eosinophils as the primary remaining cells).

#### **Prognostic assays**

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FPRL1 receptor or FPRL1 ligand expression or activity. For example, the described assays can be used to identify a subject having or at risk of developing a disorder such as a neurodegenerative disorder. Typically, a test sample is obtained from a subject and FPRL1 receptor or FPRL1 ligand is detected or activity is assayed. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Prognostic assays can be used to determine whether a subject can be administered a modality (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, food, etc.) to treat a disease or disorder associated with aberrant FPRL1 receptor or FPRL1 ligand expression or activity. Such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. The invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FPRL1 receptor or FPRL1 ligand expression or activity. In such an assay, a test sample is obtained and SHAAGtide or nucleic acid is detected (e.g., where the presence of SHAAGtide or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant FPRL1 receptor or FPRL1 ligand expression or activity).

The following examples are given to illustrate the invention and are not intended to be limiting.

## EXAMPLES

*Example 1: CK $\beta$ 8-1(25-116), like other CK $\beta$ 8 variants, stimulates intracellular calcium flux in CCR1 expressing cells.*

The human recombinant chemokines, leukotactin, three known CK $\beta$ 8 variants CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116) and a novel NH<sub>2</sub>-terminal truncated form of CK $\beta$ 8-1, CK $\beta$ 8-1(25-116) were obtained from R&D Systems (Minneapolis, MN). CK $\beta$ 8-1(25-116) was compared with the other three variants for the ability to elicit an intracellular calcium mobilization in stable human CCR1 transfected HEK239 cells. Human HEK293-CCR1 cells were prepared using Fugena 6 (Roche, IN) following the

manufacturer's protocol. The HEK-293 cell lines were maintained in DMEM with 10% FBS supplemented with 800 µg/ml G-418.

Stable expression of human chemokine receptor CCR1 in HEK293 cell was obtained as follows: full length cDNA encoding CCR1 was cloned by the polymerase chain reaction (PCR) from genomic DNA isolated from human peripheral blood cells. The PCR product was cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA) using standard molecular cloning procedures and completely sequenced to confirm identity.

Two micrograms of the CCR1/pcDNA3.1 construct were used to transfect the HEK293 cells as follows. FuGENE:DNA complex was prepared by mixing 6.0 µl FuGENE 6 reagent (Roche Molecular Biochemicals, CA) and 2 µg CCR1/pcDNA3.1 in 100 µl of serum-free medium (Hyclone, CO). After incubating for 30 minutes at room temperature, the complex was added to a 60 mm culture plate containing 0.5 - 1 X 10<sup>6</sup> cells in 10 ml DMEM medium supplemented with 10 % FBS (Hyclone, CO). After mixing, the cells were returned to the incubator to culture at 37°C for two days. At 48 hours post-transfection, Genetinin (G418) (Mediatech, Herndon, VA) was added at a final concentration of 800 µg/ml. The cells were then plated in 96-well plates at a concentration of 20,000 cells/well. After 2-3 weeks under G418 selection, stable geneticin-resistant CCR1 expressing cells were assessed for their ability to mobilize calcium in response to MIP-1α at a concentration of 1-500 nM.

Ca<sup>2+</sup> mobilization responses were performed using the intracellular ratiometric fluorescent dye, Indo-1. Cells were loaded with Indo-1/AM (3 µM; Molecular Probes, Eugene, OR) in culture medium (45 min, 20°C, 10<sup>7</sup> cells/ml). After dye loading, cells were washed once with 10 ml PBS) and resuspended at 10<sup>6</sup> cell/ml in HBSS containing 1% FBS. Cytosolic [Ca<sup>2+</sup>] release was determined using excitation at 350 nm using a Photon Technology International fluorimeter (excitation at 350 nm, ratioed dual emission at 400 and 490 nm).

With HEKCCR1-293 transfectants, CKβ8-1(25-116) and the other CKβ8 variants induced a rapid calcium flux at 100 nM. The two truncated variants CKβ8(25-99) and CKβ8-1(25-116) induced a high calcium response, while the signals generated by variants CKβ8(25-99) and CKβ8-1(1-116) were lower. None of these chemokines induced a signal with the untransfected parental HEK293 cells, demonstrating that the activity is due to CCR1 and not an endogenous receptor. The maximal receptor stimulations obtained with 100nM CKβ8(25-99) and CKβ8-1(25-

116) were equivalent to those obtained with the same concentration of the CCR1 agonist, leukotactin.

*Example 2: CCL23 variant CK $\beta$ 8-1(25-116) displays an unique activity profile in human monocyte and neutrophils that is not a CCR1 linked event.*

The human recombinant chemokines, leukotactin, MIP-1 $\alpha$ , three known CK $\beta$ 8 variants CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116) and a novel NH<sub>2</sub>-terminal truncated form of CK $\beta$ 8-1, CK $\beta$ 8-1(25-116) were obtained from R&D Systems (Minneapolis, MN). Human monocytes were generated from buffy coats (Stanford Blood Center, Palo Alto, CA) following a standard protocol. Briefly, PBMC were isolated by standard density gradient centrifugation (Ficoll-Paque-Plus, Pharmacia). Monocytes were purified using CD14 Microbeads (Miltenyi, Auburn, CA) magnetic positive selection. Human neutrophils were isolated from fresh peripheral blood from healthy individuals by gradient centrifugation on Ficoll-Hypaque (Hyclone, CA).

The activity of the CCL23 variants was tested on freshly prepared human monocytes and neutrophils using the calcium flux test described in Example 1. Although all of the chemokines stimulated some calcium release on monocytes, CK $\beta$ 8(1-99) and CK $\beta$ 8-1(1-116) showed poor activity, even at 250nM. CK $\beta$ 8(25-99) showed slightly higher calcium stimulation. However, CK $\beta$ 8-1(25-116) exhibited a unique calcium flux with extended calcium release. The maximal receptor stimulation obtained with 100 nM CK $\beta$ 8-1(25-116) was at least two fold higher than that obtained with the same concentration of leukotactin.

On neutrophils, 100 nM leukotactin induced a calcium flux but neither MIP-1 $\alpha$  nor CK $\beta$ 8(1-99), CK $\beta$ 8(25-99) or CK $\beta$ 8(1-116) induced a calcium flux. However, CK $\beta$ 8-1(25-116) induced an unique calcium release. The magnitude was much higher than observed for the same amount of leukotactin stimulation.

*Example 3: Cross-desensitization test performed on HEK293-CCR1 transfectants, monocytes and neutrophils.*

In cross-desensitization tests tests, cells were stimulated sequentially with leukotactin and then the chemokines CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116), and CK $\beta$ 8-1(25-116). On HEK293-CCR1 transfectants (prepared as in Example 1), leukotactin induced similar patterns of receptor desensitization to all variants. When

the cells were pretreated with 100 nM leukotactin, the calcium flux response to all ligands was completely inhibited.

Similar receptor cross-desensitization tests were performed using both monocytes and neutrophils (prepared as in Example 2). On monocytes, leukotactin completely desensitized the CCL23 variants, CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116). In contrast, leukotactin prestimulation did not desensitize CK $\beta$ 8-1(25-116) activity on monocytes. On neutrophils, CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), and CK $\beta$ 8-1(1-116) were inactive and prestimulation with leukotactin had no effect. However, leukotactin prestimulation did not desensitize the stimulation with CK $\beta$ 8-1(25-116).

*Example 4: CCL23 variants compete with  $^{125}\text{I}$ -MIP-1 $\alpha$  for binding to CCR1-expressing cells.*

The binding characteristics of CCL23 variants was compared in human CCR1 expressing cells. The ability of MIP-1 $\alpha$  and the CCL23 variants CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116) and CK $\beta$ 8-1(25-116) to compete with  $^{125}\text{I}$ -MIP-1 $\alpha$  binding was investigated in HEK293-CCR1 cells (prepared as in Example 1). The cells were incubated with  $^{125}\text{I}$ -labeled MIP-1 $\alpha$  (final conc.  $\sim$  0.05 nM) in the presence of unlabeled chemokine (3 hours at 4°C: 25 mM HEPES, 140 mM NaCl, 1 mM CaCl<sub>2</sub>, 5mM MgCl<sub>2</sub> and 0.2% BSA, adjusted to pH 7.1). Reaction mixtures were aspirated onto PEI-treated GF/B glass filters using a cell harvester (Packard). The filters were washed twice (25 mM HEPES, 500 mM NaCl, 1 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, adjusted to pH 7.1). Scintillant (MicroScint-10; 35  $\mu$ l) was added to dried filters and the filters counted in a Packard Topcount scintillation counter. Data were analyzed and plotted using Prism software (GraphPad Software, San Diego, CA).

Competition curves were observed with increasing concentrations of MIP-1 $\alpha$  or CCL23 variants. MIP-1 $\alpha$  gave an IC<sub>50</sub> of 0.54 nM. The CCL23 variants gave IC<sub>50</sub> values of 64 nM, 1.34 nM, 206 nM, and 112 nM, respectively. CK $\beta$ 8-1(1-116) showed 3-4 fold less potency than CK $\beta$ 8(1-99) on this transfectant for the displacement of the bound  $^{125}\text{I}$ -MIP-1 $\alpha$  from CCR1, consistent with its relatively weak affinity for CCR1. Also as expected, the truncation of CK $\beta$ 8(1-99), CK $\beta$ 8(25-

99), showed a 40-fold IC<sub>50</sub> increase. However, the IC<sub>50</sub> for CKβ8-1(25-116), the same amino acid truncated variant of CKβ8-1(1-116), is only increased one fold.

Similar binding competition tests were conducted on monocytes and neutrophils. These cells were prepared as in Example 2. Binding competition between MIP-1α on neutrophils could not be studied, since MIP-1α does not bind neutrophils. On monocytes, MIP-1α has an IC<sub>50</sub> of 0.27 nM, and CCL23 variants IC<sub>50</sub>s of 10 nM, 0.25 nM, 55 nM, and 5 nM, respectively. Overall, CKβ8(1-99) and CKβ8(25-99) showed similar IC<sub>50</sub> to that observed on HEK293-CCR1 cells. However, CKβ8-1(1-116) and CKβ8-1(25-116) showed higher MIP-1α displacement activities on monocytes, especially CKβ8-1(25-116) which was over 10 fold higher. <sup>125</sup>I-MIP-1α binding-competition data (IC<sub>50</sub>) is shown in Table 6. The IC<sub>50</sub> for each interaction was derived from non-linear least squares curve fitting.

Table 6. <sup>125</sup>I-MIP-1α Binding Competition Data for HEK293-CCR1 Transfectants and Monocytes

	HEK293-CCR1	Monocytes
MIP1α	0.54 nM	0.27 nM
CKβ8(1-99)	64 nM	10.3 nM
CKβ8(25-99)	1.34 nM	0.25 nM
CKβ8-1(1-116)	206 nM	55 nM
CKβ8-1(25-116)	112 nM	5.1 nM

*Example 5 Variant CKβ8-1(25-116) induces human monocyte and neutrophil migration with a novel migratory property.*

Migration assays were performed on monocytes and neutrophils. Human monocytes and neutrophils (prepared as in Example 2) were harvested and resuspended in chemotaxis medium (CM). The CM consisted of Hank's buffered salt solution (Gibco, MA) containing CaCl<sub>2</sub> (1 mM) and MgSO<sub>4</sub> (1 mM) with added 0.1% BSA (Sigma, St. Louis, MO). The assays were performed in 96-well ChemoTx® microplates (Neuroprobe, MA). Leukotactin, MIP-1α and chemokines (CKβ8(1-99),

CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116) or CK $\beta$ 8-1(25-116), prepared as in Example 1) were added to the lower wells (final volume 29  $\mu$ L), and 20  $\mu$ L of cell suspension ( $5 \times 10^6$  cells/mL for monocytes;  $2.5 \times 10^6$  cells/mL for neutrophils) added to the polycarbonate filters (5  $\mu$ m pore size for monocytes; 3  $\mu$ m pore size for neutrophils). After  
5 incubation for 90 min (37°C, 100% humidity, 5 % CO<sub>2</sub>), cells were removed from the upper surface of the filter by scraping. Cells that migrated into the lower chamber were quantified using the Quant cell proliferation assay kit (Molecular Probes, OR).

On monocytes, CK $\beta$ 8-1(25-99), CK $\beta$ 8-1(1-99) and CK $\beta$ 8-1(1-116) showed moderate activity at all concentrations up to 100 nM. CK $\beta$ 8-1(25-116) showed a  
10 dramatically higher activity than the other three variants at 100 nM, although its activity at 1 and 10 nM was very similar to the other variants.

The same test was performed on human neutrophils. Human neutrophils generally lacked robust response to CCR1 ligands. Consistent with the calcium flux results, none of the known CCR1 ligands including leukotactin and MIP-1 $\alpha$  was  
15 active. However, CK $\beta$ 8-1(25-116) induced a robust response at 100 nM. This magnitude is comparable to many other potent CXCR1 and CXCR1 ligands including IL-8 and GRO- $\alpha$ .

*Example 6 CK $\beta$ 8-1(25-116) is able to induce intracellular calcium flux and  
20 chemotaxis in formyl peptide receptor like 1 (FPRL1) expressing cells.*

The functional activities of CK $\beta$ 8-1(25-116) were investigated in human FPRL1-L1.2 transfectants and in native L1.2 cells. Stable expression of formyl peptide-like receptor 1 (FPRL1) in L1.2 cell was obtained as follows. Full length cDNA encoding FPRL1 was cloned, using the polymerase chain reaction (PCR), from  
25 genomic DNA isolated from undifferentiated HL-60 cells. The polymerase chain reaction product was cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA) using standard molecular cloning procedures and completely sequenced to confirm identity. Twenty micrograms of the FPRL1/pcDNA3.1 construct were linearized by digestion with BsmI (New England Biolabs, Beverly, MA) and used to transfect the murine B  
30 cell line L1.2 as follows. Twenty five million cells were washed twice and resuspended in 0.8 ml of PBS. The cells were incubated for 10 min at room temperature with the linearized FPRL1/pcDNA3.1 construct DNA and transferred to a 0.4-cm cuvette, and a single electroporation pulse was applied at 250 V, 960  $\mu$ F.

Electroporated cells were incubated for 10 min at room temperature and transferred to culture at 37°C in RPMI supplemented with 10% FCS. Geneticin (G418) was added to a final concentration of 800 µg/ml 48 h posttransfection and the cells plated into 96-well plates at 25,000 cells/well. After 2–3 weeks under drug selection, stable  
5 geneticin-resistant FPLR1 expressing cells were assessed for their ability to mobilize  $\text{Ca}^{++}$  in response to SHAAAGtide or CKβ8-1 at concentrations of 1 to 1000 nM.

A calcium flux test was performed on these transfectants using the method described in Example 1. Of the CCL23 variants, only CKβ8-1(25-116) stimulated calcium release in FPRL1 expressing cells. The synthetic peptides Trp-Lys-Tyr-Met-  
10 Val-D-Met-NH<sub>2</sub> (WKYMVm) and Trp-Lys-Tyr-Met-Val-Met-NH<sub>2</sub> (WKYMVM) (“W peptides 1 and 2”)(obtained from Phoenix Pharmaceuticals (Belmont, CA)), known non-natural ligands for FPRL1, produced a robust calcium flux. A CKβ8-1(25-116) induced calcium release was not observed in parietal cells or cells transfected with other chemokine receptors. When a CKβ8-1(25-116) induced  
15 calcium flux dose response assay was performed, an EC<sub>50</sub> of 10-20 nM was observed on these cells. CKβ8-1(1-116) showed no activity on FPRL1 expressing cells, even at 200 nM.

The ability of CKβ8-1(25-116) to elicit the migration of the FPRL1 expressing cells was examined. Test conditions were as in Example 5. Although parietal L1.2  
20 cells did not migrate in the assay, cells expressing FPRL1 migrated in a bell-shaped dose-dependent manner in response to CKβ8-1(25-116) concentrations ranging from 1 nM to 1 µM. The half-maximal cell migration was observed at 30 nM. The magnitude of the maximal response was higher than observed with the synthetic peptides WKYMVm and WKYMVM. In general, compared to the other chemokines,  
25 CKβ8-1(25-116) showed a broader bell-shaped curve in FPRL1 mediated migration. Hence, in addition to its activity on CCR1, CKβ8-1(25-116) also functions through the receptor FPRL1 expressed on monocytes and neutrophils.

*Example 7 CKβ8-1(25-116) is able to displace <sup>125</sup>I-labeled WKYMVm  
30 binding on human monocytes and FPRL1 expressing cells.*

The binding of CKβ8-1(25-116) to FPRL1 was determined by measuring the ability of CKβ8-1(25-116) to displace <sup>125</sup>I-labeled WKYMVm (<sup>125</sup>I-labeled Trp-

Lys-Tyr-Met-Val-D-Met-NH<sub>2</sub>, Perkin Elmer Life Science (Boston, MA)) from human FPRL1-L1.2 transfectants and human monocytes. Cells were incubated with 0.01 nM <sup>125</sup>I-WKYMVm in the presence of increasing concentrations of unlabeled WKYMVm or CKβ8-1(25-116) for three hours at 4 degree C. The IC50 for each interaction was derived from non-linear least squares curve fitting of the data by using Prism software (GraphPad Software).

For FPRL1-L1.2 transfectants and monocytes, competition curves were observed with increasing concentrations of WKYMVm or CKβ8-1(25-116) (Table 7). Such curves were not observed for other CCL23 variants.

Table 7 <sup>125</sup>I-labeled WKYMVm competition curves observed with WKYMVm and CKβ8-1(25-116).

	IC50	
	Human Monocytes	L1.2 FPRL1 Cells
WKYMVM	1.5nM	80nM
CKβ8-1(25-116)	31nM	196nM

*Example 8 Chemokine or SHAAptide induced calcium mobilization by Immature Dendritic Cells, Mature Dendritic Cells, Monocytes or Neutrophils.*

Human recombinant CKβ8-1(25-116) chemokine was obtained from R&D Systems (Minneapolis, MN). The peptide SHAAGtides SEQ ID NO:1 and SEQ ID NO:6 and a control protein (the reverse sequence of SEQ ID NO:1 ) were synthesized and HPLC-purified using routine techniques as described in Sambrook *et al.*, 1989, and Ausubel *et al.*, 1999.

Human monocytes were either generated from buffy coats (Stanford Blood Center, Palo Alto, CA) or from fresh blood of healthy individuals following a standard protocol. Briefly, PBMC were isolated by a Ficoll-Paque gradient centrifugation (Ficoll-Paque-Plus, Pharmacia). Monocytes were purified by CD14 Microbeads (Miltenyi) magnetic positive selection. Human neutrophils were isolated from fresh blood by dextran sedimentation and gradient centrifugation Ficoll-Paque gradient centrifugation. All cells were washed and resuspended ( $1 \times 10^7$ /ml) in RPMI medium with 10% FBS.

Immature DCs were derived by culturing CD14<sup>+</sup> monocytes in the presence of GM-CSF and IL4. Briefly, monocytes were cultured in a T-175 flask at 10<sup>6</sup> cells/ml in RPMI/10% FCS. Recombinant human GM-CSF and IL4 were added on day 0, 2, 4 and 6 to a final concentration of 1000 u/ml and 500 u/ml, respectively. Cells were  
5 harvested on day 7 as immature DCs and characterized for surface protein expression by FACS analysis. DC maturation was carried out by culturing day 6 immature DCs in macrophage-conditioned medium (MCM). Briefly, day 6 immature DCs were harvested by centrifugation and resuspended in MCM at 10<sup>6</sup> cells/ml. The medium was supplemented with 1000 u/ml of GM-CSF and 500 u/ml IL4. After three more  
10 days of culture, cells were harvested as mature DCs and characterized by surface protein expression flow cytometry.

MCM was prepared as follows: PBMCs isolated from buffy coat were incubated at 37°C in a plastic flask pre-coated with 10 mg/ml human IgG (Sigma, St Louis, MO) for 30 minutes. After 30 minutes, non-adherent cells were removed and  
15 adherent cells were washed three times with DPBS, then cultured in RPMI/10% human serum. Conditioned-medium was collected after 24 hours. TNF- $\alpha$  concentration, which is critical for DC maturation, was determined by using a TNF- $\alpha$  ELISA kit (R&D Systems, MN). The final TNF- $\alpha$  level in MCM was adjusted to 50 u/ml by mixing with RPMI/10% human serum, and was stored at -80 freezer until use.

20 Ca<sup>2+</sup> mobilization responses were performed using an intracellular ratiometric fluorescent dye, Indo-1. Cells were loaded with Indo-1/AM (3  $\mu$ M; Molecular Probes (Eugene, OR)) in culture medium (45 min, 20°C, 10<sup>7</sup> cells/ml). After dye loading, cells were washed once (10 ml PBS) and resuspended at 10<sup>6</sup> cell/ml in HBSS containing 1% FBS. Cytosolic [Ca<sup>2+</sup>] release was determined using excitation at 350  
25 nm using a Photon Technology International fluorimeter (excitation at 350 nm, ratioed dual emission at 400 and 490 nm).

The SHAAgtides SEQ ID NO:1 and SEQ ID NO:6, as well as CK $\beta$ 8-1(25-116), produced a robust calcium flux on human monocytes and neutrophils and were partially active on immature Dendritic Cells and mature Dendritic Cells. No  
30 significant calcium flux was observed with the control peptide. Since immature Dendritic Cells express high levels of CCR1, CK $\beta$ 8-1(25-116) induces Ca<sup>2+</sup> release in these cells.

*Example 9 Chemokine, SHAAGtide, and SHAAGtide truncated variants induced calcium mobilization by stable expressed FPRL1 cells.*

Stable expression of human FPRL1 in L1.2 cells was obtained as in Example 6. Calcium flux tests were conducted on the transfectants using the method described in Example 1. Chemokines (CK $\beta$ 8(1-99), CK $\beta$ 8(25-99), CK $\beta$ 8-1(1-116) and CK $\beta$ 8-1(25-116), were prepared as in Example 1. W peptides 1 and 2 were obtained as in Example 6. In addition, and the following SHAAGtide sequences and truncated variants (prepared as in Example 8) were tested:

10	CCXP1	SEQ ID NO:1
	CCXP2	SEQ ID NO:2
	CCXP3	SEQ ID NO:3
	CCXP4	SEQ ID NO:4
	CCXP5	SEQ ID NO:5
15	CCXP6	SEQ ID NO:6
	CCXP7	SEQ ID NO:7
	CCXP8	SEQ ID NO:8
	CCXP9	SEQ ID NO:9
	CCXP10	SEQ ID NO:10

20

All ligands were added in a dose response manner and the peak calcium flux response determined. Table 8 shows that CK $\beta$ 8(25-116) (SEQ ID NO:16) and certain SHAAGtides induced calcium mobilization in FPRL1 transfectants. However, CK $\beta$ 8(1-116), which does not contain a free SHAAGtide N-terminal, did not give significant mobilization. The data also indicates that the N-terminal of the SHAAGtide is important for its activity in FPRL1 transfectants. Those SHAAGtides having a truncated N-terminal gave greatly reduced calcium mobilization. SHAAGtides having a truncated or substituted C-terminal did not exhibit the same loss in activity as was observed after truncation of the N-terminal.

30

Table 8 – Induction of Calcium Flux in FPRL1 L1.2 Cells. (IC50 – where no IC50 value is listed, the sequence showed low or no significant activity.)

	IC 50
SEQ ID NO:1	150nM
SEQ ID NO:2	>50 $\mu$ M
SEQ ID NO:3	68nM
SEQ ID NO:4	-
SEQ ID NO:5	7.4nM
SEQ ID NO:6	38nM
SEQ ID NO:7	-
SEQ ID NO:8	45nM
SEQ ID NO:9	-
SEQ ID NO:10	-
SEQ ID NO:13 - CK $\beta$ 8(1-99)	-
SEQ ID NO:14 - CK $\beta$ 8(25-99)	-
SEQ ID NO:15 CK $\beta$ 8(1-116)	-
SEQ ID NO:16 CK $\beta$ 8(25-116)	11nM
W peptide 1	0.7nM
W peptide 2	<0.1 $\mu$ M

5

*Example 10 Chemotactic activity of Chemokines, SHAAGtide and SHAAGtide truncated variants on FPRL1-L1.2 cells.*

The chemotactic activity of chemokines (CK $\beta$ 8(1-99) and CK $\beta$ 8(25-99), SHAAGtides (SEQ ID NO:1 and SEQ ID NO:2) and W peptides 1 and 2 (obtained as  
 10 in Example 6) on FPRL1-L1.2 cells was determined in migration assays. The chemokines were prepared as in Example 1. The SHAAGtide sequences SEQ ID NO:1 and SEQ ID NO:2 were prepared as in Example 8. FPRL1-L1.2 cells were prepared as in Example 6.

The migration assays were performed in 96-well ChemoTx® microplates  
 15 (Neuroprobe) using the protocol described in Example 5. Both SEQ ID NO:1 and

CKb8-1(25-116) migrated the transfectants, indicating that they are functional for this receptor.

*Example 11 Chemotactic activity of Chemokines, SHAAGtide and SHAAGtide  
5 truncated variants on human monocytes and neutrophils.*

The chemotactic activity on human monocytes and neutrophils of chemokines:  
CKβ8(1-99), CKβ8(25-99), CKβ8(1-116) and CKβ8(25-116); W peptides 1 and 2  
and the SHAAGtide sequences SEQ ID NO:1 and SEQ ID NO:2 was determined in  
migration assays. The above peptides were prepared as in previous examples. The  
10 migration assays were performed in 96-well CHEMOTX® microplates (Neuroprobe)  
using the protocol described in Example 5. Both SHAAGtide SEQ ID NO:1 and  
CKb8-1(25-116) produced migration of both neutrophils and monocytes.

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## CLAIMS

1. An isolated protein or polypeptide comprising a N-terminal sequence having at least 80% identity to SEQ ID NO:1, excluding the sequence of SEQ ID NO:16.
- 5 2 The isolated protein or polypeptide of claim 1, wherein the protein or polypeptide is a ligand for FPRL1.
3. The isolated protein or polypeptide of claim 1, wherein said N-terminal sequence has at least 90% sequence identity to SEQ ID NO:1.
4. The isolated protein or polypeptide of claim 1, wherein said N-terminal  
10 sequence has at least 95% sequence identity to SEQ ID NO:1.
5. The isolated protein or polypeptide of claim 1, wherein said N-terminal sequence has 100% sequence identity to SEQ ID NO:1.
6. The isolated protein or polypeptide of claim 1, wherein said N-terminal sequence is SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5 or SEQ ID NO:6.
- 15 7. An isolated polypeptide at least 80% identity to SEQ ID NO:1.
8. The isolated polypeptide of claim 7 having at least 90% identity to SEQ ID NO:1.
9. The isolated polypeptide of claim 7 having at least 95% identity to SEQ ID NO:1.
- 20 10. The isolated polypeptide of claim 7 having a sequence of SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5 or SEQ ID NO:6.
11. An isolated nucleic acid comprising a nucleic acid sequence comprising at least 80% identity with SEQ ID NO:20.
12. The isolated nucleic acid of claim 11 comprising at least 90% identity to SEQ  
25 ID NO:20.
13. The isolated nucleic acid of claim 11 comprising at least 95% identity to SEQ ID NO:20.

14. The isolated nucleic acid of claim 11 comprising at least 99% identity to SEQ ID NO:20.
15. An isolated nucleic acid having a sequence complementary to the nucleic acid of claim 11.
- 5 16. The isolated nucleic acid of claim 11 comprising SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or SEQ ID NO:30.
17. The isolated nucleic acid of claim 15 comprising SEQ ID NO:20.
18. The isolated nucleic acid of claim 15 comprising SEQ ID NO:25.
19. An antibody that specifically binds to the peptide of claim 1.
- 10 20. A fusion protein comprising a non-SHAAGtide polypeptide fused to the peptide of claim 1.
21. A kit comprising a pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier, and a syringe.
22. A method of identifying a FPRL1 receptor antagonist comprising:
  - 15 contacting a cell expressing a FPRL1 receptor with a protein or polypeptide comprising a N-terminal sequence having at least 80% identity to SEQ ID NO:1; wherein the receptor is stimulated;
  - contacting the receptor with a candidate antagonist compound; and
  - detecting to the FPRL1 receptor.
- 20 23. The method of claim 22, wherein the candidate compound is an antibody, peptide, nucleic acid or small molecule.
24. The method claim 22, wherein the cell is a neutrophil, monocyte, T-  
25 lymphocyte or dendritic cell.

Figure 1 *Reported FPRL1 ligands*

Ligands	Calcium Flux (EC50)	Migration (EC50)	Binding (EC50)
<u>Endogenous Ligands:</u>			
Lipoxin A4	?	?	2-10 nM to H <sup>3</sup> -LPX
Serum amyloid A	>0.2 uM	>0.2 uM	250 nM to [ <sup>125</sup> I]-SSA
$\beta$ -Amyloid <sub>(1-42)</sub>	>2 uM	10 uM	
Prion Peptide PrP <sub>(108-126)</sub>	>2 uM	25-50 uM	
LL-37 <sup>1</sup>	>5 uM	5-10 uM	
D2D3 <sub>(88-274)</sub> <sup>2</sup>	?	0.1 nM (on 283-FPRL1) <sup>3</sup>	83 nM to [ <sup>125</sup> I]-D2D3 <sub>(88-274)</sub>
<u>Viral &amp; Bacterial Encoded:</u>			
T21/DP107 (HIV gp41)	>0.5 uM	>0.5 uM	
HIV gp120 <sub>(414-434)</sub>	5-10 uM	5-10 uM	
H. pylori peptide, Hp <sub>(2-20)</sub> <sup>4</sup>	10 uM		
rMLP	>5 uM		
<u>Non-natural Ligands:</u>			
WKYMVm	0.1-1 nM	0.1-1 nM	10 nM to [ <sup>125</sup> I]-W Peptide
WKYMVM	1-10 nM	1-10 nM	>10 nM to [ <sup>125</sup> I]-W Peptide
MMK-1	low nM	low nM	

*Notes:*

1. Human cathelicidin-derived antibacterial peptide, LL-37.
2. Urokinase plasminogen activator (uPA), D2D3(88-274).
3. In neutrophils, neither uPA nor D2D3(88-274) induce calcium flux.
4. Helicobacter pylori peptide, Hp(2-20).

CKβ8 (1-99) RVTKDAETEFMMSKLPLENPVLLD-----RFHATSADCCISYTPRSIP  
 CKβ8 (25-99) RFHATSADCCISYTPRSIP  
 CKβ8-1 (1-116) RVTKDAETEFMMSKLPLENPVLLDMLWRRKIGPQMTLSHAAGFHATSADCCISYTPRSIP  
 CKβ8-1 (25-116) MLWRRKIGPQMTLSHAAGFHATSADCCISYTPRSIP  
 MIP1δ QFINDAETELMMSKLPLENPVVLN-----SFHF-AADCCTSYSQSIP  
 Leukotactin SFHF-AADCCTSYSQSIP  
 MIP1α SLAADTPTACCFYSYTSRQIP

CKβ8 (1-99) CSLLLESYFETNSECSKPGVIFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN  
 CKβ8 (25-99) CSLLLESYFETNSECSKPGVIFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN  
 CKβ8-1 (1-116) CSLLLESYFETNSECSKPGVIFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN  
 CKβ8-1 (25-116) CSLLLESYFETNSECSKPGVIFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN  
 MIP1δ CSLMKSYFETSSCSKPGVIFLTKKGRQVCAKPSGPGVQDCMKKLPYSI  
 Leukotactin CSLMKSYFETSSCSKPGVIFLTKKGRQVCAKPSGPGVQDCMKKLPYSI  
 MIP1α QNFIDYFETSSQCSKPGVIFLTKRSRQVCADPSEEWVQKYVSDLE

Figure 2.  
 CKβ8(1-99) SEQ ID NO:13 MIP-1δ SEQ ID NO:17  
 CKβ8(25-99) SEQ ID NO:14 Leukotactin SEQ ID NO:18  
 CKβ8(1-116) SEQ ID NO:15 MIP-1α SEQ ID NO:19  
 CKβ8(25-116) SEQ ID NO:16

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26339

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 5/00, 15/12, 15/63; C07K 14/00, 16/00

US CL : 435/69.1, 320.1, 325; 530/350, 387.1, 388.22

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 320.1, 325; 530/350, 387.1, 388.22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
EAST MEDLINE BIOSIS EMBASE CAPLUS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/32481 A1 (BANDMAN et al.) 17 October 1996 (17.10.96) see entire document.	1-24
Y	US 5,504,003 A ( LI et al.) 02 April 1996 (02/04/96), see entire document.	1-24
Y	WO 98/14582 A1 (HUMAN GENOME SCIENCES, INC.) 09 April 1998 (09.04.98) see pages 1-176.	1-24

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier application or patent published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\*

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\*

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\*

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\* & \*

document member of the same patent family

Date of the actual completion of the international search

26 September 2002 (26.09.2002)

Date of mailing of the international search report

10 DEC 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26339

### Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 1-24  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Please See Continuation Sheet
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
- Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

PCT/US02/26339

## Continuation of Box I Reason 2:

Claims 1-24 are unsearchable to the extent that they require reference to the specified sequences from the sequence listing. Because Applicant failed to furnish a machine-readable copy of the sequence listing, no meaningful search of the sequences per se can be carried out by this Authority. However, the subject matter of the claims has been searched to the extent possible with reference to the balance of the description.